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中国东南沿海四种拟菱形藻生长特性与毒素检测研究

Growth characteristics and toxin determination of four species of *Pseudo-nitzschia* from Southeast China Sea

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中文摘要

本论文对分离自中国东南沿海的 4 种（15 株）拟菱形藻进行了形态学，不同培养条件下的生长与毒素检测的研究，初步探讨了中国海域拟菱形藻未发现可检出毒素的原因；对可产毒种类—多列拟菱形藻（*Pseudo-nitzschia multiseries*, 加拿大株系)进行了形态学、生长和毒素检测与比较，分析了不同株系多列拟菱形藻毒素产量的差异和不同生长周期对毒素产生的影响；对分离自中国东南沿海的 13 种（21 株）其它羽纹纲硅藻进行了软骨藻酸（Domoic Acid, DA）检测研究，分析了中国海域贝类体内发现毒素的可能原因。取得的主要结果如下：

利用微吸管法从中国东南沿海不同水域分离得到 4 种(15 株)拟菱形藻，利用光学显微镜和透射电镜进行了形态学研究，鉴定为尖刺拟菱形藻(*P. pungens*)、尖细拟菱形藻（*P. cuspidata*）、多纹拟菱形藻（*P. multistriata*）和巴西拟菱形藻（*P. brasiliana*），其中除巴西拟菱形藻外，其它三种在国外均有产毒报道。

以细胞密度、叶绿素 a 含量及生长速率三种因子为指标，对 5 种拟菱形藻进行了不同条件培养下生长的研究与比较，结果显示，在常规培养条件下，不同株系间在生长上表现出显著差异，验证了来源地不同的同种拟菱形藻间地理差异的存在；对同一株系，不同条件下的培养也显示出显著差异的存在，说明外界环境因子的改变，会影响拟菱形藻的生长特性。

通过设置不同的光周期和硅、磷营养盐限制条件，对从中国东南沿海分离得到的 4 种拟菱形藻进行了常规培养和诱导培养实验，采用高效液相色谱法(HPLC)对各个生长周期的培养藻液进行了检测，均未发现可检出 DA 存在，说明本实验所用的 4 种拟菱形藻在上述培养条件下，均不能产生可检出 DA。

对 Bates Stephen (加拿大 Bedford 海洋研究所) 提供的加拿大有毒拟菱形藻 *P. multiseries* 进行了常规条件培养，比较研究了与中国不同藻种的生长差异，进行了毒素检测和产量分析，验证了 DA-HPLC 方法的可行性，并为下一步对中国水域拟菱形藻与国外产毒拟菱形藻之间进行对比，寻找其不产毒的原因提供了前期工作基础。

对实验室现有的 13 种 (21 株) 分离自中国东南沿海的羽纹纲硅藻进行了 DA-HPLC 检测，均未发现可检出 DA 存在，说明本实验室藻种库现有的 21 株羽纹纲硅藻可能不能产生 DA。

关键词：拟菱形藻；生长；软骨藻酸检测.

Abstract

This dissertation mainly focuses on the studies on four species of genus *Pseudo-nitzschia* isolated from Southeast China Sea and the studies include the morphological study, growth study underd different conditions, toxin (Domic Acid) determination and the primary exploration of the reasons why the *Pseudo-niztschia* distributed in China Sea cannot produce DA. The morphological study, growth and toxin (Domic Acid) determination on the six strains of *P. multiseries* (from Canada) were also carried out. The different DA yields between the *P. multiseries* strains and different cell cycle are analyzed. In addition, the toxin (Domic Acid) determination were also carried out on thirteen pennate species (twenty-one strains) isolated from China coastal sea area and the possible reasons of the toxin in seashells were discussed. The results are as followed:

Thirteen strains of *Pseudo-nitzaschia* isolated from different locations in Southeast China Sea by microtube method. These thirteen strains *Pseudo-nitzschia,* are identified as four species that are *P. pungens, P. multiserita, P. cuspidata* and *P. brasiliana* by light microscope and electron microscope. Except the *P. brasiliana*, the rest have been reported to be toxin producers.

Taking the cell density, Chl*a* and growth rate as the indicators, four species of *Pseudo-nitzschia* that are isolated from different locations of Southeast China Sea were studied by being cultivated respectively under normal conditions and different inducing conditions. The results suggested that the strains isolated from different locations have differences in growing conditions under the normal cultivating conditions, which supported that the same species from different geographical areas do have differences. And as for the same species, the differences do exist under the different cultivating conditions, which showed that the changes of environmental factors can influence the growth characteristics.

Deficiency of silica and phosphorus, different photoperiods were set as the inducing cultivation. According to the HPLC measurement, no DA was determined under the set cultivating conditions, which lead to the conclusion that these four

species of *Pseudo-nitzschia* under the study cannot produce DA either under the normal conditions or inducing conditions.

Under the normal conditions, the growth study between the strains, the toxin (Domic Acid) determination and DA yield analysis were also carried out on the *P. multiseries* provided by the Bates Steven, (Bedford institution of oceanography, Canada). The results approve the testing method applied in this study is feasible and accurate. And what is more, the result provide the rudimentary data for further study on the comparison between the DA producers and no DA producers, and the laid the foundation for exploration of the reasons why the *Pseudo-niztschia* distributed in China Sea area cannot produce DA.

DA-HPLC was also carried out on 21 strains which belong to 13 species of pennate diatoms isolated from China Sea. The results showed that 21 species of pennate diatoms cannot produce tested DA.

Key words: *Pseudo-nitzschia;* growth; domoic acid analysis.